

Center Reflections

A bimonthly publication highlighting activities at the W.M. Keck Foundation Center for Molecular Structure

California State University Fullerton

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**ISABELLA KARLE:
JOHN BRYDEN DISTINGUISHED
LECTURER**

CSU Fullerton

On February 10, 2000 CMoLS and the Department of Chemistry and Biochemistry at CSU Fullerton proudly hosted Dr. Isabella Karle as the University's John Bryden Distinguished Lecturer. The lecture series is named for Professor Emeritus John Bryden, who was the Department's founding faculty member, a physical chemist and a crystallographer.

Isabella Karle is senior scientist and head of the Naval Research Laboratory's (NRL) x-ray diffraction section in the Laboratory for the Structure of Matter. Karle pioneered new ways to study the three-dimensional structure of molecules, making use of both X-ray and electron diffraction. Her method for determining essentially equal-atom crystal and molecular structures by x-ray analysis transformed analyses formerly characterized by tedious frustration and abortive efforts to ones that now involve systematic processes. All the present computerized programs for x-ray structure analyses are based on Karle's fundamental work known as the *Symbolic Addition Procedure*. Her procedures have been adopted



worldwide and have contributed to the explosive output of crystal structure analyses. More than 10,000 analyses are now published annually compared to about 150 annually in the early 1960s.

In addition to developing the widely used method for structure determination from x-ray diffraction, Karle has identified and determined the structures of a number of complex substances of considerable chemical and biomedical significance. She applied the direct method of phase determination to the elucidation of molecular formulas and conformations of steroids, alkaloids, frog toxins, photorearrangement products, and particularly peptides. Karle also has taught the new analytical techniques to a large

number of students and researchers, greatly enhancing their capabilities.

Karle received her B.S. degree in chemistry in 1941; her M.S. degree in physical chemistry in 1942; and her Ph.D. degree in physical chemistry in 1944, all from the University of Michigan. She also holds four honorary doctoral degrees. At Michigan, she met her husband, also a Ph.D. student in chemistry. After graduation, both Karles worked in Chicago on the Manhattan Project and then returned to Ann Arbor. At the end of World War II, they went to Washington, where they landed jobs at the Naval Research Lab in 1946, which was willing to hire them both.

"The problem was that we were looking for academic jobs, and we couldn't get work in the same city," she says, citing as reasons universities' anti-nepotism policies, bias against women, and not enough research funding in smaller towns for both of them.

Her husband, Jerome Karle, now chief scientist at NRL's Laboratory for the Structure of Matter, began working on so-called *direct methods* for analyzing crystal structures, the foundation mathematics for determining phase information from x-ray diffraction patterns. Along with his collaborator, Herbert A. Hauptman of the Medical Foundation of Buffalo, Jerome Karle received the Nobel Prize in chemistry in 1985 for contributions to crystallography.

Isabella Karle stresses that it was her husband, Jerome, who developed theories about X-ray crystallography that she pursued. "I'm an experimentalist," she says. Her earlier research was in electron diffraction. She taught herself X-ray crystallography from textbooks and then developed techniques for studying crystals, which led to a greater understanding of their three-dimensional nature and the effect on physical and biological properties. In the 1950s,

Isabella Karle sought practical applications for her husband's mathematical theories. In 1963, she introduced the *Symbolic Addition Procedure*, which revolutionized the types and complexity of problems that can be solved by analyzing crystal structures. "What she came up with is now being used by crystallographers throughout the world," says Drake Eggleston, an associate fellow at SmithKline Beecham in Philadelphia. "We used to have to spend an inordinate amount of time trying to understand structures. Now our understanding occurs faster."

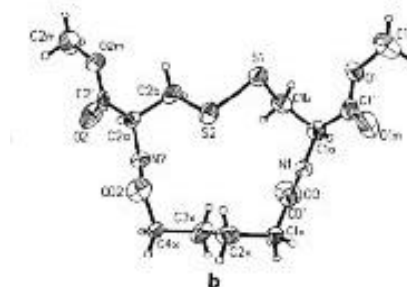
Over the years she has received many honors. Among them are: the American Chemical Society's Hillebrand Award (1970) and Garvan Medal (1976); Lifetime Achievement Award (1986) from Women in Science and Engineering; Secretary of the Navy Distinguished Achievement in Science Award (1987); the 1988 Gregory Aminoff Prize from the Swedish Royal Academy of Sciences; the 1991 Paul Ehrlich Prize from the National Institutes of Health; the Vincent du Vigneaud Award at the Gordon Conference on Peptides (1992); the Franklin Institute's prestigious Bower Award and Prize in Science (1993); and the National Academy of Sciences Award in Chemical Sciences (1995). In addition, in 1989 she was inducted into the Michigan Women's Hall of Fame. She has been a member of the National Academy of Sciences since 1978. She has also served as president of the American Crystallographic Association.

In 1995, Karle was selected to receive the National Medal of Science. She was honored for "developing a method for determining essentially equal-atom crystal and molecular structures by x-ray analysis, thereby having a profound effect on the practice of organic and biological chemistry." The National Medal of Science is the highest scientific honor bestowed by the President of the United States.

During her recent visit, Karle expressed her concerns that crystallography is not often taught in the undergraduate curriculum, and that its importance as a fundamental discipline of modern chemistry is underappreciated. Karle was extremely impressed by the activities at CMoIS and that crystallography has been incorporated into the undergraduate curriculum at many levels. She has agreed to serve as an advisor to CMoIS.

Karle's lecture in February focused on some of her more recent work with peptide nanotubes. A family of peptaibols found in spores in the soil perform antibiotic functions by transporting potassium ions through cell membranes. Single crystals have been prepared of two members of this family, the 16-residue peptides zervamicin and anti-amoebin, and their structures established to a high resolution by X-ray diffraction analyses. Aspects of the structures discussed by Karle were the nature of the severely bent helices assumed by the peptides, the assembly of several helical peptimolecules that form the ion channel, the environment of the ion channel in a surround of long chain alcohol veral helical peptide molecules that form the ion channel, the environment of the ion channel in a surround of long chain alcohol molecules that mimic membrane material, and the gating mechanism that regulates the passage of one potassium ion per opening of the gate.

Karle also described the single crystal x-ray structures of several cystine-based peptide nanotubes.



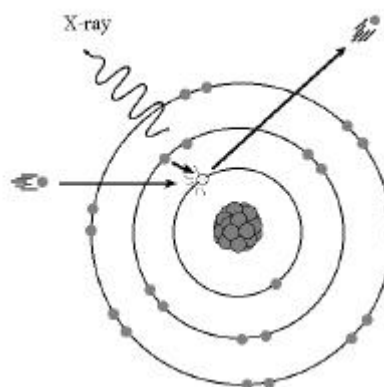
Molecular structure of cyclobisamide.

Journal of Organic Chemistry **1999** 64, 9230-9240.

X-rays and Laboratory X-ray Sources

CMoIS

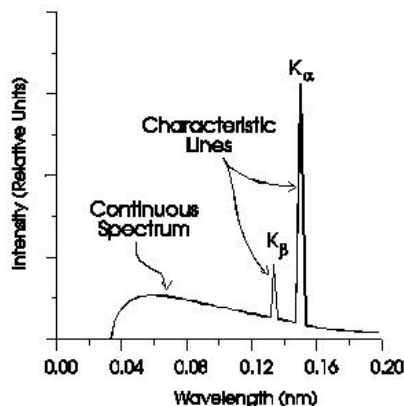
X-rays are electromagnetic radiation with wavelengths 10^{-7} - 10^{-11} m (1000 - 0.1Å). *Characteristic radiation* is produced when an incoming electron hits an electron of a target atom. The electron of the target atom is ejected. An electron from an outer



shell fills the hole. The difference in binding energy between the two shells is emitted in the form of an X-ray, whose energy (wavelength) is characteristic of the atom and the two shells. A useful conversion factor between wavelength and energy units is

$$\lambda(\text{nm}) = 1.24 / E(\text{keV})$$

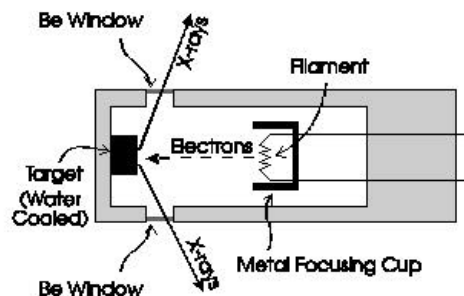
Conventional x-ray sources may produce radiation of different energies (wavelengths). Anodes typically found in the laboratory have Mo and Cu targets. The spectrum of x-rays has a dome shape appearance, with peaks corresponding to the characteristic radiation of the target atom. Shown below is the x-ray spectrum for Cu.



X-rays for use in diffraction experiments are generated by an electron beam impinging on a metallic target, the **anode**. The electrons are generated by heating a filament, the **cathode**, usually made of tungsten because of its high melting temperature, which provides enough thermal energy for some electrons to escape from the metal. The electrons are then accelerated by a potential, a process similar to that used to generate the electron beam in a TV or computer monitor.

The geometry of a sealed tube x-ray source is shown in the figure in the next column. The high voltage is applied to the metal **focusing cup**, which focuses the electrons into a broad beam that strikes the anodic target several centimeters away. The kinetic energy of each electron that strikes the target is transformed into an x-ray when each electron strikes the target and collides with an electron in an atom. The x-ray tube is evacuated during manufacturing so that the electrons don't lose energy by collisions

with air molecules as they travel from the filament to the target. In addition, the x-ray tube is surrounded by a jacket that circulates cooling water to dissipate the heat that is generated. Sealed tube x-ray sources tubes are limited to 1-3 kW power (kW is the product of the kV x mA). A typical setting for a 2.0 kW tube is 50 kV x 40 mA.



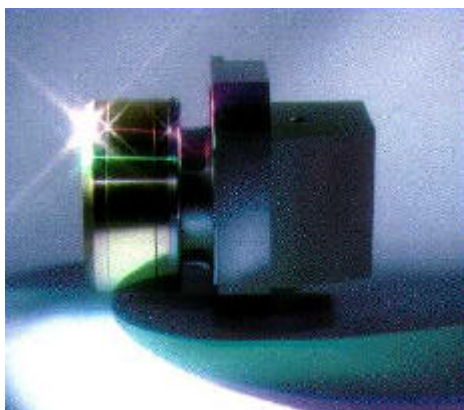
The x-rays leave the x-ray generating tube by passing through a thin beryllium metal "window". Beryllium is selected as the window material because it absorbs fewer x-rays for a given window thickness than any other solid element except Li, and the reactivity of Li prevents its use as a window material in x-ray tubes. The primary x-ray beam is made monochromatic using either metal foils, crystal monochromators or focussing mirrors.

It can be seen from the geometry of the x-ray tube that a cone-shaped (diverging) beam of x-rays emerges from the x-ray tube through the Be window. For most diffraction experiments, a narrow beam with little divergence is needed. Even though x-rays are electromagnetic radiation like visible light, no known material can act as an x-ray lens to focus the divergent beam. The best that we can do is to collimate the divergent beam from the x-ray tube using small apertures that absorb all but a narrow portion of the original beam. However, using a small aperture to produce a collimated x-ray beam also reduces the intensity of the beam (because more of the original x-rays are

blocked off within the collimator). Thus, collimation of the x-ray beam involves a trade-off between a more parallel beam with lower intensity vs. a more divergent beam with higher intensity.

The lifetime of a typical sealed tube varies between 3000 and 20,000 hours, depending on type and use. Replacement cost is ~\$4000.

When higher power is required in the laboratory, a rotating anode is used. As seen in the figure below, the anode consists of a 'wheel' plated with a metallic target material, which is mounted on an axis that is rotating at ~ 4500 rpm during operation. Rotating the anode and cooling it internally with circulating water dissipate additional heat. This type of x-ray source can be run at higher power, producing higher intensity radiation. A disadvantage over the sealed tube is that a rotating anode source requires continuous pumping to keep the vacuum at the required level.



Rotating anodes must have their ferro-fluidic seals replaced regularly, and these anodes must periodically be replated with their target material. Furthermore, the newer direct drive rotating anodes must have worn bearings replaced. Thus, the lifetime of a rotating anode varies between 6 months and a few years depending on type and use. A new Cu or Mo anode costs ~\$10,000. Refurbished anodes cost ~\$6500. Fine filaments last approximately 1000 hours and cost \$450.

In the next issue, we will describe and discuss the various types of detectors available.

Crystals: What If You Have an Evil Twin?

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You have diligently recrystallized the product of a lengthy synthesis. Or, you have struggled through numerous random screens and crystal trays, and at last you have discovered crystals of your protein. Eureka! But soon thereafter (or maybe after some time and serious crystallographic work), you determine that your crystals have evil twins.

Crystal twinning usually arises for good structural reasons. Giacovazzo¹ defines twins as "regular aggregates consisting of crystals of the same species, joined together in some definite mutual orientation". To describe a twin, we then need an orientation of the different species, or twin domains, relative to one another (called the twin law), and the fractional contribution of each species component. The twin law is usually expressed as a matrix, which transforms the *hkl* indices of one species into the other.

The following cases of twinning are commonly encountered in the laboratory:

- (a) In twinning by merohedry, where reciprocal lattices exactly coincide, lower symmetry trigonal, tetragonal, hexagonal or cubic Laue groups may look more like the corresponding higher symmetry Laue groups, assuming the c-axis unique except for cubic.
- (b) Twinned orthorhombic with **a** and **b** axes approximately equal may emulate tetragonal symmetry.
- (c) Twinned monoclinic cells with a **b** angle near 90 degrees may emulate orthorhombic symmetry.

- (d) Twinned monoclinic cells with **a** and **c** axes approximately equal and a **b** angle near 120 degrees may emulate hexagonal symmetry.

Twinned crystals are not always obvious, although examination of your crystals under an optical microscope between crossed polarizers may provide evidence of twinning. Examination of your crystals under an optical microscope is good practice in general. In addition, you may detect non-merohedral twinning by direct observation of two partially overlapped reciprocal lattices. Twinning is likely when a structure cannot be solved by routine methods, despite what appear to be good data.

There are several characteristic crystallographic warning signs for twinning. Not all of these will be evident in a particular experiment, but if you observe several of the following phenomena, you should seriously consider the possibility of twinning. (f) through (j) are typical in cases of non-merohedral twinning, where reciprocal lattices do not overlap exactly.

- (a) The metric symmetry is higher than the Laue symmetry. The twin operator belongs to the symmetry of the lattice point and not to the Laue group.
- (b) The R_{merge} value for the higher symmetry Laue group is only slightly higher than that for the lower symmetry Laue group.
- (c) The mean value for $|E^2 - 1|$ is much lower than the expected value of 0.736 for the non-centrosymmetric case. The reason for this is that if the crystal consists of two twin domains, and every reflection has contributions from both domains, it is unlikely that both contributions will have very high intensities or that both will have very low intensities. Thus, the intensities are distributed with fewer extreme values.

- (d) Unusual or impossible systematic absences are observed.
- (e) The Patterson function is physically impossible.
- (f) There appears to be one or more unusually long axes.
- (g) Refinement of a cell is problematic.
- (h) Reflections may show variable profiles: some sharp, others split.
- (i) $\langle F_o^2 \rangle / \langle F_c^2 \rangle$ is systematically high for low intensity reflections.
- (j) Reflections considered 'most disagreeable' have $F_o \gg F_c$.

SHELXL² implements the twin refinement method of Pratt et al.³ and Jameson⁴. SAINT+ implements the *TWIN* Programs, a package of four programs that find autoindexing solutions for non-merohedrally twinned samples. One of these programs, *TWINDX* is an extensive modification of an algorithm first developed by Robert Sparks⁵. Unit cell parameters and orientation matrices are generated which are used to determine the relative orientations of the twin components. Finally, component numbers and indices are generated for overlapped and unoverlapped reflections.

1. Giacovazzo, C. (1992). Editor. *Fundamentals of Crystallography*. Oxford University Press.
2. Herbst-Irmer, R. and Sheldrick, G.M. (1998) *Acta Cryst.* B54, 443-449.
3. Pratt, C.S., Coyle, B.A. and Ibers, J.A. (1971) *J. Chem. Soc.* Pp. 2146-2151.
4. Jameson, G.B. (1982). *Acta Cryst.* A38, 817-820.
5. Sparks, R.A. (1982). *Computational Crystallography*. Edited by D. Sayre. Clarendon Press: Oxford, pp. 1-18.

Websites of Interest

UCL BSM CATH Dictionary of Homologous Superfamilies

<http://www.biochem.ucl.ac.uk/bsm/dhs/index.html> CATH is a classification of protein domains.

UCLA Bioinformatics Homepage

<http://www.bioinformatics.ucla.edu/> UCLA Bioinformatics is a completely interdisciplinary initiative involving over 30 faculty members representing 10 departments. Faculty from departments as disparate as pediatrics and mathematics have joined forces to both develop mathematics and elucidate the mysteries of biology.

NDB Biological Structure Server

<http://ndbserver.rutgers.edu/> The NDB Biological Structure Resource (BSR) has been developed by the Nucleic Acid database Project at Rutgers University.

CRYSTOOL <http://www-structure.llnl.gov/crystool/crystool.htm> See Volume 1, Issue 6 of *Center Reflections*.

Growing Crystals that Will Make Your Crystallographer Happy

<http://rocket.chem.ualberta.ca/xray/links.html>. See Volume 1, Issue 6 of *Center Reflections*

Crystallography 101 <http://www-structure.llnl.gov/Xray/101index.html> - See Volume 1, Issue 3 of *Center Reflections*.

Upcoming Events

March 16-17, 2000: **Bruker Area Detector User's Group Meeting (BADUG) or "Reciprocal Space Camp"**, Texas A&M University (TAMU), Bryan-College Station, TX. <http://www.chem.tamu.edu/services/crystal>

March 26-30, 2000: **American Chemical Society National Meeting**, San Francisco, CA. <http://www.acs.org/meetings/sanfran2000/>

April 15-18, 2000: **Experimental Biology 2000 FASEB Meeting and Scientific Exposition**, San Diego, CA. <http://www.faseb.org/eb2000>

May 5-6, 2000: **CSU Systemwide Research Competition**, Cal Poly Pomona.

May 14-19, 2000: **8th International Conference on the Crystallization of Biological Macromolecules (ICCBM 8)**, Sandestin, FL. <http://ICCBM8.cmc.uab.edu/>

July 22-27, 2000: **American Crystallographic Society Annual Meeting**, St. Paul, MN. <http://nexus.hwi.buffalo.edu/ACA/ACA-Annual/StPaul/StPaul.html>

August 5-9, 2000: **The Protein Society Annual Symposium**, San Diego, CA. <http://www.faseb.org/meetings/protein00/>

August 20-24, 2000: **American Chemical Society National Meeting**, Washington D.C. <http://www.acs.org/meetings/washington2000/>

October 25-28, 2000: **American Chemical Society Western Regional Meeting**, San Francisco, CA. <http://www.mcs.csuhayward.edu/~wwwchem/>

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<http://www-structure.llnl.gov/scaurcon99/cmols2.html>

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Staff Scientist: We're looking for someone.
Contact us!

Interim technical assistance is being provided by Jeff Madrid, a research student at CSUF.